AD-A131 315 STUDIES ON THE TRANSOVARIAL TRANSMISSION OF PHLEBOTOMUS FEVER VIRUSES IN SANDFLIES(U) YALE UNIV NEW HAVEN CONN SCHOOL OF MEDICINE R B TESH FEB 82 DAMD17-80-C-0178 1/1

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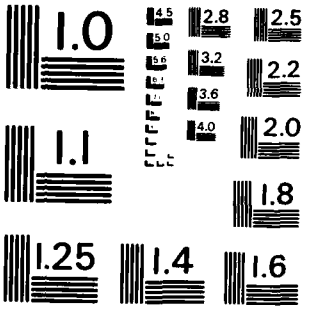

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Robert B. Tesh, M.D.

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During the past year, laboratory colonies of 3 sandfly species (Phlebotomus papatasi, L. longipalpis and L. anthophora) were established. Rio Grande virus (family Bunyaviridae, genus Phlebovirus) replicated in L. anthophora and was transovarially transmitted by this species after intrathoracic inoculation. Multiplication and transvarial transmission of Pacui virus was also demonstrated in L. longipalpis. Multiplication of Naples sandfly fever virus was shown in P. papatasi. Initial attempts to establish a sandfly cell culture are promising, but growth of the cells is slow.

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Contract No.: DAMD 17-80-C-0178
Name of Contractor: Yale University (School of Medicine)
Principal Investigator: Robert B. Tesh, M.D.
Date of Report: February 1982

A. Establishment of Sandfly Colonies

During the past year, closed breeding colonies of 3 sandfly species (Phlebotomus papatasi, Lutzomyia longipalpis and L. anthophora) were established in our laboratory. The P. papatasi strain was started by Govind Modi, formerly of the National Institute of Virology, Poona, India. This strain is of Indian origin and most of the females are autogenous. Mr. Modi joined the project in January 1981 as a graduate research assistant and now maintains our sandfly colonies.

The L. longipalpis colony was obtrained from Louis C. Rutledge, Letterman Army Institute of Research, San Francisco. This strain is of Brazilian origin.

The L. anthophora strain was obtained from Richard Endris, Institute of Food and Agricultural Sciences, Gainsville, Florida. This colony was started from parents collected in South Texas. The colonies of P. papatasi, L. longipalpis and L. anthophora are doing well and we currently produce about 2,000 adults of each species per month. The limiting factor in our production is the number of man hours required to care for the sandflies. The life cycle of these insects at 25°C takes approximately 5 to 6 weeks. The sandflies (adults and larvae) must be checked daily to insure that they have adequate food and moisture. Consequently, rearing the sandflies is extremely labor intensive.

During the past year, we also received eggs of two other sandfly species, L. flaviscutella and L. trapidoi, from Brazil and Panama, respectively. Both species did poorly in the laboratory and were lost after 2 generations. In view of our success with the other 3 species and the work required to maintain them, we deided to confine the virus studies to P. papatasi, L. longipalpis and L. anthophora.

B. Experimental Virus Infection of Sandflies

1. Rio Grande virus. Rio Grande virus is a member of the genus Phlebovirus. Its presumed vector is L. anthophora. Approximately 100 L. anthophora females were infected by intrathoracic inoculation with this virus. Table 1 shows the growth of Rio Grande virus in the infected sandflies. Mean virus titers in the infected insects increased more that 4 logs during the first seven days after inoculation. Thereafter, the virus titer appeared to stabilize at about $10^{4.5}$ $TCID_{50}$ per insect.

Some of the infected female L. anthophora were fed on a normal hamster 2 and 3 days post-inoculation, and their subsequent eggs (first ovarian cycle only) were collected. The F_1 progeny hatching from these eggs were

adults and tested for the presence of Rio Grande virus. A total of [illegible] (34/62) of the F_1 adults were infected with the virus. The sex ratio of the infected off spring was similar (50% and 59.3% for males and females, respectively), indicating that transovarial transmission of the virus occurs at fairly high levels.

2. Pacui virus. A smaller number of L. longipalpis females were inoculated with Pacui virus. Preliminary studies indicate that this virus replicates in L. longipalpis, although specimens have not yet been titrated to construct a growth curve. A sample of 66 F_1 progeny obtained from infected female parents was tested for the presence of Pacui virus. Thirty percent (20/66) of the F_1 offspring were infected. Further studies are planned with this virus-sandfly model. The lower transovarial transmission rate obtained with Pacui virus may be due to the fact that L. longipalpis is not the natural vector. The presumed natural vector of this virus is L. flaviscutella, a species we have so far been unable to colonize.

3. Naples sandfly fever virus. This virus, which is transmitted by a number of different sandfly species, is one of the major causes of phlebotomus fever in the Old World. Approximately 100 P. papatasi were inoculated with Naples virus and a sample of 5 insects each was taken daily for titration. Growth of the virus following inoculation is shown in Table 2. Mean virus titers in the infected flies increased about 2 logs within 7 days after inoculation to a maximum titer of about $10^{4.0}$ PFU, results similar to those obtained with Rio Grande virus in L. anthophora. Studies are now in progress to determine if this virus is transovarially transmitted.

C. Sandfly Cell Culture

Attempts have been made to establish a sandfly cell culture. L. longipalpis eggs were surface sterilized, ruptured, and transferred to MM/VP_{12} growth medium at 28°C. Initially, a number of cells attached to the surface of the container. Some of these have divided and grown; however, growth has been very slow. (This is the usual pattern for primary insect cell cultures). We are presently maintaining the cell cultures on growth medium in the hope that one or more clones will begin to proliferate more rapidly and can be used to start a continuous cell line.

D. Publications

1. Tesh, R.B., Peters, C.J. and Meegan, J.D.: Studies on the antigenic relationship among phleboviruses. Am.J.Trop.Med.Hyg. 31:149-155, 1982.

Table 1

Growth of Rio Grande virus in Lutzomyia anthophora after intrathoracic inoculation

Day post-inoculation	Number/Number infected/sampled	Range of titers in infected flies*	Mean titer in infected flies*
0 (immediately after inoculation)	5/5	$10^{0.4}$ - $10^{1.1}$	$10^{0.6}$
1	5/5	$10^{0.7}$ - $10^{1.7}$	$10^{1.3}$
2	5/5	$10^{1.7}$ - $10^{3.4}$	$10^{2.5}$
3	5/5	$10^{1.7}$ - $10^{3.7}$	$10^{2.6}$
4	4/5	$10^{2.9}$ - $10^{3.1}$	$10^{3.1}$
5	4/5	$10^{3.4}$ - $10^{5.0}$	$10^{4.1}$
6	-	-	-
7	5/5	$10^{4.3}$ - $10^{5.7}$	$10^{5.0}$
8	2/2	$10^{4.0}$ - $10^{4.3}$	$10^{4.2}$
9	2/2	$10^{4.5}$	$10^{4.5}$
10	1/2	$10^{4.8}$	$10^{4.8}$

*Tissue culture infectious dose$_{50}$/per insect.

Table 2

Growth of Naples sandfly fever virus
in Phlebotomus papatasi after intrathorocic inoculation

Day post inoculation	Number /Number infected/sampled	Range of titers in infected flies*	Mean titer in infected flies*
0	5/5	2.0 - 2.7	2.2
1	5/5	2.0 - 2.6	2.3
2	5/5	2.8 - 3.4	3.0
3	5/5	3.0 - 4.2	3.7
4	5/5	3.0 - 4.3	3.5
5	5/5	3.0 - 4.0	3.6
6	5/5	4.0 - 4.2	4.1
7	5/5	3.8 - 4.5	4.2

*Titers expressed as $\log_{10}$ of plaque forming units per insect.

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